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Parvovirus B19-associated acute myocarditis in paediatric patients

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ABSTRACT:

— ***Background:*** Viral aetiology of acute myocarditis has been proposed by many authors. Parvovirus B19 (PVB19) has recently emerged as a potential pathogen associated with myocarditis in adults. In this report, we describe four paediatric patients with acute myocarditis due to PVB19 infection.

— ***Patients and Methods:*** Four children (three males and one female), aged between 7 and 18 months who were suffering from acute myocarditis were enrolled. Heart biopsies were collected, after acquiring written informed consent. Blood, serum and endomyocardial biopsy (EMB) were tested for most common viral agents. PVB19 DNA was evaluated by nested PCR analysis.

— ***Results:*** Each patient presented with mild anemia and fever. Myocarditis was associated with the presence of PVB19 DNA in EMB in all cases; in fact, PVB19-specific DNA sequences were repeatedly found in all heart biopsies by nested-PCR, amplifying NS viral region. No other cardiotropic viruses were found.

— ***Conclusions:*** PVB19 infection could be responsible for a variety of acute and chronic diseases, including acute myocarditis, in paediatric cases. As a consequence, it is critical to investigate the presence of PVB19 in any cases of myocarditis of unknown aetiology in children.

— ***Key words:*** Myocarditis, Parvovirus B19, Antibodies, Molecular diagnosis, Paediatrics patients.

INTRODUCTION

Myocarditis is usually due to viral infections and spontaneous recovery is the most common outcome[1]. Occasionally active myocarditis[2] can lead to death[3,4]. Five to 10% of all patients may develop chronic dilated cardiomyopathy[5,6]. Inflammatory processes induced by viral or bacterial infections are considered as one of the major pathogenetic mechanisms of myocardial diseases. Although the pathogenetic pathways of progression to myocardial failure are not fully understood, persistent viral infections, alone or in combination with autoimmune dysregulation[7,8], have been postulated as potential mechanisms. Myocardial virus persistence is frequently observed in patients with non-ischemic heart disease. A variety of cardiotropic viruses may induce myocarditis, with enterovirus and adenovirus as the most frequent causative agents in children and adolescents. In patients with clinically suspected myocarditis, myocardial enterovirus persistence has been demonstrated in 40% of cases, 56% of whom actively replicating[9]. However, parvovirus B19 (PVB19) has recently emerged as another potential pathogen among adult patients with clinical signs of acute or chronic myocarditis[10-12]; in acute myocarditis, PVB19 has been demonstrated in endomyocardial biopsies (EMB) in 71% of patients[13]. Nevertheless, in adult patients the detection of PVB19 DNA in EMB remains incompletely understood and its prognostic value controversial[14].

After the development of PCR methods, PVB19 infection has been linked to a variety of acute and chronic diseases, such as chronic arthropathy[15,16], acute fulminant liver failure in children[17], and acute myocarditis[18,19]. However, because of the limited data available, the etiopathogenetic mechanisms of PVB19 in inflammatory heart disease remain unclear.

In this study, we report four cases of PVB19-associated acute myocarditis in paediatric patients which occurred in Eastern Sicily, Italy.

PATIENTS AND METHODS

Four children, three males and one female, aged between 7 and 18 months, were diagnosed with signs of acute myocarditis. They have been hospitalized in different cardiological departments. Serum, blood and biopsies were

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obtained from all children, after acquiring written informed consent. Heart biopsies were collected into sterile DNAse free tubes for DNA analysis. All samples were immediately frozen at -20°C until used and tested at the Clinical Virology Unit of the University of Catania, Italy.

IgM and IgG titres to PVB19 were evaluated by commercial enzyme immunoassay (Biotrin, Dublin, Ireland). These are characterized by a prominent IgG immune response against the viral protein (VP) C-terminal linear epitopes within VP2, usually detectable only within the first months after acute infection. IgG and IgM to Coxsackievirus type B from B1 to B6 were titrated by an indirect immunofluorescence assay[20]. Cytomegalovirus (CMV) and Epstein Barr (EBV) (Vidas, BioMérieux, Marcy-l'Etoile, France) antibodies were measured using commercial Enzyme Linked Fluorescent Assay (ELFA) kits.

For DNA analysis, myocardial tissue fragments of approximately 2 mm were digested by 12 U of proteinase K (Roche Applied Science, Mannheim, Germany) in 200 µl of ATL lysis buffer (Qiagen, Hilden, Germany) at 55°C; DNA extraction was performed with a DNeasy Tissue Kit (Qiagen, Hilden, Germany). Two hundred microliters of blood sample from each child was extracted with High Pure Viral Nucleic Acid Kit (Roche Applied Science, Mannheim, Germany). Detection of PVB19 DNA was performed by nested PCR analysis amplifying NS viral region by primer sequences from the published nucleotide sequence of B19 virus (accession no. AF162273; GenBank, Bethesda, MD, USA) and carry out by the Primer Express program. First round primers included PV1: (nucleotides 1611-1632) and PV2: (nucleotides 1767-1788), which span a 194-bp segment. Second round (nested) primers were PV3: (nucleotides 1627-1649) and PV4: (nucleotides 1710-1731), which span a 103-bp segment. Amplification was done in 50 µl reaction volumes of 67 mM Tris (pH 8.8)-16.6 mM $(NH_4)_2SO_4$- 6.7 mM $MgCl_2$-10 mM mercaptoethanol- 6.7 µM EDTA- 50 pmol of each primer-5 U/µl of Taq polymerase overlaid with mineral oil. Taq polymerase was from Bioline (London, UK). The reaction was carried out in a GeneAmp® PCR System (Applied Biosystems 9700, Foster City, CA, USA), with a reaction profile of 4 min at 95°C and 35 cycles of 1 min at 95°C, 1 min at 55°C and 1 min at 72°C, followed by a final extension of 5 min at 72°C. Initial amplification with external primers was followed by reamplification with inner primers (30 cycles). Positive and negative controls were included in each test. The products of the reaction were run on a 2% agarose-gel (Invitrogen, Waltham, MA, USA) in Tris-borate-EDTA buffer.

RESULTS

All infants were born at term by vaginal delivery and their birth weight ranged from 3000 to 3350 g. Clinical and virological findings for patient 1-4 with PVB19-associated myocarditis are listed in Tables 1 and 2.

Each patient initially had mild anemia. Myocarditis was associated with PVB19 DNA in the blood and serum in patients 1, 3 and 4, whereas it was associated with PVB19-specific IgM antibodies in patient 1, at the onset of disease. In contrast, PVB19 IgM was not detected in the other cases (Table 1). Among the patients whose serological tests were IgM negative and IgG positive, the diagnosis of PVB19 infection was confirmed by PCR with blood specimens (Table 2). All available EMBs were positive for PVB19 by nested PCR analysis. Paired serum samples from all 4 patients were negative for IgM and IgA antibodies to Coxsackie B1 to B6 viruses and echoviruses. Moreover, there were no significant values in the IgG titers detected in all patients included in this study. In addition, these samples were negative for IgM and IgG antibodies specific to cytomegalovirus and Epstein-Barr virus (EBV). CMV, EBV, Adenovirus and Enterovirus were negative in all samples available, except in serum of patient 1 and blood of patient 3 that were positive for Enterovirus and EBV nucleic acids, respectively (Table 2).

Table 1. Demographic and clinical characteristics of 4 patients with Parvovirus B19 (PVB19) infection.

	Patients			
Variable	**1**	**2**	**3**	**4**
Age	7 months	10 months	12 months	18 months
Sex	F	M	M	M
PVB19 IgG serostatus at disease onset	+	+	+	+
PVB19 IgM serostatus at disease onset	+	-	-	-
PVB19 PCR result	+	+	+	+
Anemia	YES	YES	YES	YES
Lowest Hgb level, mg/dl	ND	11.6	8.4	ND
Leukopenia	ND	NO	NO	ND
WBC count, $x10^3$	ND	9.9	12.1	ND
Thrombocytopenia	YES	NO	YES	YES
Symptoms	Fatigue, dyspnea, weakness, fever	Fever, headache, dyspnea	Fever, tachycardia, weakness	Fatigue, dyspnea, weakness, fever
Treatment	ND	IVIG	Digital, ACE-inhibitors, diuretics	Digoxin, furosemide, captopril, ceftriaxone

NOTE. IVIG, intravenous immunoglobulin; ND, not done; Hgb, hemoglobin.

Table 2. Virological findings into different samples of 4 paediatrics patients.

	Blood	Serum	EMB	Liquor	Urine
Patient 1	CMV – EBV – PVB19 +	ENT + PVB19 + Adeno –	ENT – PVB19 + CMV – EBV – Adeno -	\\	\\
Patient 2	CMV – EBV – PVB19+	ENT – PVB19 – Adeno –	ENT - PVB19 + CMV – EBV – Adeno –	CMV – HSV1 – HSV2 – EBV – PVB19 – ENT – Adeno –	EBV – PVB19 – Adeno – CMV –
Patient 3	CMV – EBV + PVB19 +	ENT – PVB19 + Adeno –	ENT – PVB19 + CMV – EBV Adeno –	\\	\\
Patient 4	CMV – EBV – PVB19 +	ENT – PVB19 + Adeno	\\	\\	\\

CMV, Cytomegalovirus; HSV1, herpes simplex virus 1; HSV2, herpes simplex virus 2; EBV, Epstein Barr virus; PVB19, parvovirus B19; ENT, Enterovirus; Adeno, Adenovirus; EMB, endomyocardial biopsy.

DISCUSSION

PVB19 tropism depends on its binding to cellular surface by the P blood group antigen globoside (Gb4)[21,22]. The P antigen is expressed on certain human cells such as erythrocytes, erythroblasts, megakaryocytes, endothelial cells, placenta, fetal liver and myocardial cells[23]. PVB19 has been demonstrated as the etiological agent of several diseases, including erythema infectiosum[9,12], hydrops fetalis and fetal death[15-17], transient aplastic crisis in patients with various forms of anemia[12,13], and chronic anemia in immunocompromised patients[24]. The particular tropism of this virus for the myocardium could explain its role in acute myocarditis. The research of PVB19 genome in EMB may provide further diagnostic information, as well as discriminate between autoimmune and viral myocarditis[25].

Several authors[18,19,26] have postulated the role of PVB19 in myocarditis. The diagnosis is based on the detection of PVB19 DNA in EMB by PCR or by *in situ* hybridation. The use of serology does not help clinicians to reach a proven diagnosis and it is difficult to interpret. Moreover, in contrast with other viruses such as enterovirus, adenovirus and cytomegalovirus, PVB19 has not always been included in the guidelines of acute myocarditis. On the contrary, PVB19 has recently emerged as one of the most important agents that could be linked to myocarditis in previously healthy children[27-30]. In the case reported by Zack et al[29], cardiac inflammation was attributed to PVB19, even though the virus was not found in the myocardium but only in the tracheal biopsy. Papadogiannakis reported on a case of fulminant myocarditis associated with PVB19 that was detected by electron microscopy observation of myocardial tissue and PCR; in that case, PVB19 serology was not significant since it was positive for IgG and negative for IgM[28]. The potential mechanism of the physiopathology of PVB19 myocarditis is still not fully understood. Nevertheless, some authors suggest a probable indirect role of PVB19 in myocyte damage. PVB19 infects endothelial cells of myocardial vessels inducing endothelial dysfunction and migration of inflammatory cells into the myocardial interstitium[31]. Molecular viral analyses were performed using PCR in all samples of the four children; PVB19-specific DNA sequences were repeatedly found in all biopsies by nested-PCR, amplifying NS viral region. No other cardiotropic viruses, specifically enterovirus, adenovirus, cytomegalovirus, and EBV, were found by PCR or RT-PCR assays performed on the above-mentioned samples. Only in case 1, the association between PVB19 and disease could be proven by serological data, being positive for both IgG and IgM, along with the positivity for PVB19 DNA in the biopsy. Although the patient tested positive for IgG for enterovirus in serum, EBM was negative for enteroviruses DNA.

In all paediatric cases, PVB19 was detected in different specimens from the same patient at the same time. Since patients 1, 2, and 3 had myocarditis in the same period of time and they were all from the same small geographic area, an outbreak was suspected. Taken together, these four cases provide convincing evidence of the involvement of parvovirus B19 in severe myocarditis. These cases, developing myocarditis during the course of systemic PVB19 infection, were confirmed by detection of viral genome in myocardium and serum for patient 1, 3 and 4.

CONCLUSIONS

We report four cases of parvovirus-associated myocarditis in previously healthy children. Serologic and PCR data provided strong evidence that our patients had a parvovirus infection at the time of onset of their illness. PVB19 could be responsible for otherwise negative myocarditis and

therefore further studies are needed to improve the clinical and diagnostic knowledge on PVB19 myocarditis, as well as more detailed diagnostic algorithms.

CONFLICT OF INTERESTS:
The Authors declare that they have no conflict of interests.

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